Data Summary and Review on the Acute Toxicity of AE C656948 (Fluopyram) to Freshwater Invertebrates - *Daphnia* sp.

PMRA Submission Number {.....} EPA MRID Number 47372324

Data Requirement:	PMRA Data Code	9.3.2
	EPA DP Barcode	353315
	OECD Data Point	{…….}
	EPA MRID	47372324
	EPA Guideline	850.1010; 72-2

Test material: **AE C656948** **Purity:** 94.7%
Common name: Fluopyram
Chemical name: IUPAC: N-{2-[3-chloro-5-(trifluoromethyl)pyridine-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name: Not reported
CAS No.: Not reported
Synonyms: BCS development number 3000314431

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	080302	

CITATION: Bruns, E. 2006. Acute Toxicity of AE C656948 (tech.) to the Waterflea *Daphnia magna* in a Static Laboratory Test System. Unpublished study performed by Bayer CropScience AG (Development, Ecotoxicology), Monheim, Germany. Laboratory report number EBGMP 046; GLP study identification number E 320 3077-2. Study sponsored by Bayer CropScience AG (Portfolio Management, Project Management/Project Planning). Study completed on September 25, 2006.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of AE C656948 to *Daphnia magna* was studied under static conditions. Daphnids were exposed to nominal concentrations of 0 (negative and solvent controls), 3.05, 4.88, 7.81, 12.5 and 20 mg a.i./L; mean-measured concentrations were <0.1029 (negative and solvent controls), 2.9, 4.6, 7.0, 11.0 and 17.0 mg a.i./L. Mortality and sublethal effects were assessed daily. The 48-hour EC_{50} and NOAEC values were >17 mg a.i/L and 17 mg a.i/L, respectively, based on a lack of immobilization. No sublethal effects were observed among any daphnids in the negative or solvent control groups, or among any animals in any treatment groups exposed to AE C656948.

Based on the results of this study, AE C656948 would be classified as practically nontoxic to *Daphnia magna* on an acute toxicity basis up to the concentration tested in this study, in accordance with the classification system of the U.S. EPA.

This study is classified as [scientifically sound or unsound] and {does or does not} satisfy guideline requirements for an acute toxicity study with freshwater invertebrates.

Results Synopsis

Test Organism Age (e.g., 1st instar): 1st instar (<24 hours old)
Test Type (Flow-through, Static, Static Renewal): Static

NOAEC: 17 mg a.i./L Probit Slope: N/A
EC_{50}: >17 mg a.i./L
Endpoint(s) Affected: None

I. REPORTED MATERIALS AND METHODS

GUIDELINE FOLLOWED: The method used in this study followed the recommendations of the U.S. EPA FIFRA § 72(2); OECD Guideline 202 (Guideline for Testing of Chemicals, *Daphnia* sp., Acute Immobilization Test) (adapted April 13, 1984); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982); EEC Directive 92/69/EEC/EWG, part C.2 (1992); Canadian PMRA Ref.: DACO 9.3.2; EU Council Directive 91/414/EEC (1991); OPPTS 850.1010; and JMAFF 12 Nousan No. 8147 (2000).

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the current OECD Principles of Good Laboratory Practice (GLP) and with the current Principles of Good Laboratory Practice according to Annex 1 of the German chemical law (ChemG), dated June 20, 2002 [except for the screening work for contaminants in the dilution water]; the USEPA-FIFRA Good Laboratory Practice Standards (40 CFR Part 160); and the GLP standards of the Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF, 11 Nousan No. 6283 from October 1, 1999) [with the exception that recognized differences exist between the GLP principles/standards of OECD and the GLP principles/standards of FIFRA and JMAFF]. The testing facility "BCS-Development, Ecotoxicology" has been inspected and certified as working in compliance with the Principles of Good Laboratory Practice by the competent authorities (GLP certificate reference: VA-1-31.11.60.04, 4th April 2003). The analytical test site "BCS-Development, Residues, Operator and Consumer Safety" has been inspected and certified as working in compliance with the Principles of Good Laboratory Practice by the competent authorities (GLP certificate reference VI-3-31.11.91.01, January 29, 2004).

A. REPORTED MATERIALS:

1. Test material AE C656948

Description: Not reported

Lot No./Batch No. : 08528/0002

Purity: 94.7%

Stability of compound under test conditions: Recoveries of exposure concentrations of AE C656948 determined from the test solutions at 48 hours ranged from 85-97% of nominal and 102-112% of 0-hour concentrations, indicating that the test chemical was stable in solution up to the concentration tested in this study.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: The test chemical, AE C656948, was stored at room temperature.

Physicochemical properties of AE C656948.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Species: *Daphnia magna*

Age at test initiation: First instar (<24 hours old)

Source: Bayer laboratory stock

B. REPORTED STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: The study author reported that previously conducted solubility tests identified values around 16 mg a.i./L as the practical limit of solubility.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details
Acclimation	
Period:	Not reported
Conditions: (same as test or not)	Same
Feeding:	Three times weekly with living cells of the green algae *Desmodesmus subspicatus*; daphnids not fed during study
Health: (any mortality observed)	No ephippia or dead animals were observed in the cultures
Duration of the test	48 hours
Test condition	
Static/flow-through	Static
Type of dilution system for flow-through method.	N/A
Renewal rate for static renewal	N/A
Aeration, if any	Test (dilution) water was reportedly aerated at 20°C for at least 48 h prior to start of the study. Test solutions were not artificially aerated during exposure.
Test vessel	
Material: *(glass/stainless steel)* Size: Fill volume:	Glass beakers 100 ml 50 ml

Source of dilution water	The study author reported that artificial water (type M7) medium was prepared using deionized water by adding mineral salts. Immediately before use in the study, the vitamin components were reportedly added using a separately deep-frozen stored stock solution.

Water parameters	
Hardness	13 German degrees (=231 mg/L as $CaCO_3$)
pH	8.1-8.2
Dissolved oxygen	7.5-8.4 mg/L
Temperature	18-22°C
Total Organic Carbon	<2 mg/L
Particulate matter	Not reported
Metals	Not detected (<0.1 or <1 µg/L)
Pesticides	Not detected (<0.01 or 0.05 µg/L)
Chlorine	<0.01 mg/L
Number of replicates	
Negative control:	6
Solvent control:	6
Treatments:	6

Number of organisms per replicate Negative control: Solvent control: Treatments:	 5 5 5
Treatment concentrations Nominal: Mean-measured:	 0 (negative and solvent controls), 3.05, 4.88, 7.81, 12.5 and 20.0 mg a.i./L (factor=1.6) <0.1029 (negative and solvent controls), 2.9, 4.6, 7.0, 11.0 and 17.0 mg a.i./L
Solvent (type, percentage, if used)	Dimethylformamide; 100 µg/1000 ml test water
Lighting	16 hours light:8 hours darkness; illuminated by "cool white" fluorescent bulbs at a light intensity of 1500 lux (maximum)
Stability of chemical in the test system	Stable. Measured concentrations of AE C656948 in the freshly prepared test solutions at test initiation ranged from 80-96% (mean: 88%) of nominal. Measured concentrations of aged test solutions after the 48-hour exposure period ranged from 90-98% (mean: 94%) of nominal.
Recovery of chemical Level of Quantitation Level of Detection	 5 µg/L 1.7 µg/L
Positive control {if used, indicate the chemical and concentrations}	N/A
Other parameters, if any	N/A

2. Observations:

Table 2: Observations

Criteria	Details
Parameters measured including the sublethal effects	Immobility and sublethal effects
Observation intervals	24 and 48 hours
Were raw data included?	Yes
Other observations, if any	N/A

II. REPORTED RESULTS:

A. REPORTED MORTALITY:

Cumulative mortality was 0% after 48 hours among all daphnids in the <0.1029 (negative and solvent controls), 2.9, 4.6, 7.0, 11.0 and 17.0 mg a.i./L treatment groups.

Table 3: Effect of AE C656948 on Mortality of *Daphnia* sp.

Treatment (mg a i./L) Mean-measured and (Nominal)	No. of organisms	Observation period			
		24 Hours		48 Hours	
		No Dead	% mortality	No Dead	% mortality
Control	30	0	0	0	0
Solvent control (DMF; 0.1 mg/L)	30	0	0	0	0
2.9 (3.05)	30	0	0	0	0
4.6 (4.88)	30	0	0	0	0
7.0 (7.81)	30	0	0	0	0
11.0 (12.5)	30	0	0	0	0
17.0 (20.0)	30	0	0	0	0
NOAEC	20 mg a.i./L				
LC_{50}	>20 mg a.i./L				
Positive control, if used Mortality: LC_{50} NOAEC:	N/A				

B. REPORTED SUBLETHAL TOXICITY ENDPOINTS:

No sublethal effects were observed among any daphnids in the 0 (negative and solvent controls), 2.9, 4.6, 7.0, 11.0 or 17.0 mg a.i./L treatment groups after 48 hours.

Table 4: Effect of AE C656948 on Immobility - *Daphnia* sp.

Treatment (mg a.i./L) Mean-measured and (Nominal)	Observation period			
	24 Hours		48 Hours	
	end-point	% affected	end-point	% affected
Control	Immobility	0	Immobility	0
Solvent control (DMF; 0.1 mg/L)	Immobility	0	Immobility	0
2.9 (3.05)	Immobility	0	Immobility	0
4.6 (4.88)	Immobility	0	Immobility	0
7.0 (7.81)	Immobility	0	Immobility	0
11.0 (12.5)	Immobility	0	Immobility	0
17.0 (20.0)	Immobility	0	Immobility	0
NOAEC	20 mg a.i./L			
LOAEC	Could not be determined			
EC_{50}	>20 mg a.i./L			
Positive control, if used % sublethal effect: EC_{50}	N/A			

C. REPORTED STATISTICS:

The solubility of the test material was reportedly limited at the highest nominal test concentration of 20 mg a.i./L. An EC50 value could not be calculated by the study author because there were no sublethal effects or immobilization. The study author did not perform statistical analysis and based toxicity values on the nominal test concentrations.

III. REVIEWER'S EVALUATION

A. DEVIATIONS FROM GUIDELINES:

1. The fluid volume of 10 ml per animal was exceeded.
2. No transition-period was integrated into the light-dark cycle.
3. Particulate matter was not included in the dilution water analysis.
4. Acclimation period was not reported.
5. The test vessels (100 ml glass beakers) and the fill volume (100 ml) were less than the minimum EPA

recommended test vessel size of 250 ml with fill volume of 200 ml.

6. Hardness measured during the definitive toxicity test (231 mg/L as $CaCO_3$) exceeded the recommended maximum level of 180 mg/L as $CaCO_3$.

B. OTHER STUDY DEFICIENCIES: None.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Due to a lack of immobility, the reviewer did not perform statistical analysis to determine the 48-hour EC_{50} value. Therefore, the EC_{50} and NOAEC values were determined based on the observational data; the reviewer calculated mean-measured concentrations and these are reported in the Executive Summary and Results sections of this DER (See Appendix I).

NOEC: 17 mg a.i./L
EC_{50}: >17 mg a.i./L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

D. ADDITIONAL REVIEWER COMMENTS:

No residues of the test item were detected in samples from the untreated water control.

The study author reported that the expected EC_{50} concentration lies above the maximum limit of the water solubility (16 mg a.i./L), so it was not useful to prepare concentrations up to an expected EC_{50} level.

The in-life portion of the definitive toxicity test was conducted from August 1-4, 2006.

E. CONCLUSIONS:

This study is/is not scientifically sound and is thus acceptable/unacceptable. The 48-hour EC_{50} and NOAEC values were >17 mg a.i./L, respectively. Based on the results of this study, AE C656948 would be classified as practically nontoxic to *Daphnia magna* on an acute toxicity basis up to the limit of water solubility, in accordance with the classification system of the U.S. EPA.

Results Synopsis

NOAEC: 17 mg a.i./L Probit Slope: N/A
EC_{50}: >17 mg a.i./L
Endpoint(s) Affected: None

IV. REFERENCES:

Baird, D.J. et al. (1991) A Comparison Study of Genotype Sensitivity to Acute Toxic Stress Using Clones of *Daphnia magna* STRAUS. Ecotoxicological and Environmental Safety 21, 257-265.

Elendt, B.P. and Bias, W.R. (1990) Trace Nutrient Deficiency in *Daphnia magna* Cultured in Standard Medium for Toxicity Testing. Effects of the Optimisation of Culture Conditions on Life History Parameters of *D. magna*. Water

Research 24 (9), 1157-1167.

Bruns, E. (2006) Acute Toxicity of Potassium Dichromate (p.a. grade) to the Waterflea *Daphnia magna*. Bayer AG unpublished report ID.: Reference 01/2006.

APPENDIX I: REVIEWER'S CALCULATION OF MEAN-MEASURED CONCENTRATIONS

nominal concentration	0-hour mean-measured	48-hour mean-measured	mean-measured	% of nominal	% of 0-hour
3.05	2.91	2.98	2.945	96.6	102.4
4.88	4.53	4.72	4.625	94.8	104.2
7.81	6.74	7.28	7.01	89.8	108.0
12.5	10.4	11.5	10.95	87.6	110.6
20	16	18	17	85.0	112.5